

BIOSYNTHESIS OF DEXTRAN

PART II:- PRODUCTION AND CHARACTERIZATION OF DEXTRAN

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ABSTRACT

Production of dextran by Leuconostoc mesenteroides using sucrose and allied products was studied. Brown sugar was found to be the best substrate (yield of dextran 24.0% on sugar basis). Urea increased the yield of dextran by 16.6% and was the most efficient source of nitrogen. Supplementation of medium with Mn^{++} and Fe (upto 100 ppm) and with Zn^{++} and Mg^{++} (upto 150 ppm) improved the yield of dextran.

Three fractions of dextran with molecular weight ranging from 30861 to 65000 and degree of polymerisation from 190 to 401 were obtained on fractional precipitation with 95% ethanol. Periodate oxidation indicated the presence of 1-6, 1-4 and 1-3 type linkages with 1-6 link being dominant (92-97%) in all the fractions.

INTRODUCTION

Microbial production of dextran has been extensively studied by many workers¹⁻⁵. Jeanes et al¹ characterized and classified dextrans from ninety six strains of bacteria. Mehre² studied the production of low molecular weight dextran from a strain of Streptococcus (DS 50). Behrens et al³ reported a semi-continuous method for the production of dextran from Leuconostoc mesenteroides using 20% sucrose as the substrate for dextran production. Suzuki⁴ studied the effect of maltose and minerals on the biosynthesis of dextran from sucrose. Dextran like polysaccharide synthesized by a strain of Lactobacillus casei has been reported by Hammond⁵ who demonstrated the production of polysaccharide in cell-free extract of bacteria.

Chemical nature and physical characteristics of dextran have also been studied by various workers⁶⁻⁸.

Present paper reports the microbial production of dextran from Leuconostoc mesenteroides using indigenous raw materials (sucrose "shukkar" jaggary etc.) as substrates. Physicochemical characteristics of the dextran produced have also been reported.

MATERIALS AND METHODS

1. Bacterial Culture:

Leuconostoc mesenteroides was isolated from sugar-cane juice and was identified according to the "Diagnostic Tables of Cowan and Steel"⁹. Morphological and biochemical characteristics of the culture were reported previously.⁹

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2. Sucrose and Allied Products:

Sucrose, "Shukkar" and jaggary (gur) were purchased from the local market.

EXPERIMENTAL

Preparation of Inoculum:

50 mls of inoculum medium (Table-I) were taken in 250 ml conical flask and autoclaved at 120°C for 35 minutes. A loopful of bacterial culture from agar slant was transferred under aseptic conditions to inoculate the flasks. The flasks were incubated at 28°C for 48 hours without shaking.

TABLE-I

Composition of Inoculum Medium

Sucrose	43%
Potassium chloride	0.3%
Yeast extract	0.1%
Water	300 ml
pH	6.8 - 7.0

Fermentation:

Fermentation medium was the same as the inoculum medium except that different carbohydrates i.e. sucrose "shukkar" and jaggary were used in 15% concentration. 40 ml of fermentation medium was sterilized in 250 ml conical flasks at 120°C for 15 minutes. Each flask was inoculated aseptically with 10 ml of inoculum. The flasks were incubated at 28°C upto 120 hours. Progress of fermentation during incubation was determined by estimating dextran after every 24 hours intervals according to Jeanes et al¹.

Effect of Nitrogen Sources on the Production of Dextran:

To study the effect of different nitrogen sources, fermentation medium containing 15% Brown sugar was supplemented with variable concentrations (0.2 - 1.0%) of Ammonium sulphate, Ammonium chloride, Ammonium nitrate and Urea.

Effect of Different Cations on the Production of Dextran:

Fermentation medium containing 15% brown sugar was supplemented with variable concentrations (providing 100 - 500 ppm of each cation) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

Isolation and Purification of Dextran:

Dextran from fermentation broth of bacterial culture was precipitated and purified for the study of physico-chemical characteristics according to the method of Jeanes *et al*¹. Three fractions were obtained by fractional precipitation with 95% ethanol.

ANALYTICAL

Physical Properties

(i) Viscosity:

Viscosity of dextran solutions (1% Aq. solution) was determined with Ostwald viscometer¹⁰. Relative viscosity was converted into intrinsic viscosity assuming that density of solution equals to density of water.

Intrinsic viscosity:
$$\frac{\text{Log of relative viscosity}}{\text{Concentration of dextran solution}}$$

(ii) Specific Rotation:

Specific rotation of each dextran fraction was determined with 1% solution (in 1N NaOH) by polarimeter as described by Jeanes *et al*¹.

(iii) Molecular weight:

It was determined according to the method of Meyer et al¹¹.

Chemical Analysis:

Moisture and ash were determined by A.O.A.C. methods¹². Reducing sugars of substrates were determined by Benedicts method¹³. Non-reducing sugars (as sucrose) were determined by Abbe-refractometer calibrated to % substrates and nitrogen of dextran fractions was determined by Micro-Kjeldhol method¹⁴.

Periodate Oxidation of Dextran:

Periodate oxidation of each dextran fraction was carried according to the method reported by Rankin and Jeanes¹⁵.

RESULTS AND DISCUSSION

1) Effect of Sucrose and other Allied Products on the Production of Dextran from *Leuconostoc Mesenteroides*.

Figure 1 shows the effect of sucrose and other allied products on the production of dextran from *Leuconostoc mesenteroides*. It is evident from the figure that brown sugar gave the maximum yield (24%) when compared with other products. Yield with jaggary sample B was the lowest (15%). The better yield with brown sugar seems to be due to the high ash contents of this sugar (Table-II) which might have effected the dextran synthesis. This fact was further supported by the supplementation of fermentation medium with minerals which indicated a positive influence on the yield of dextran (Fig.III). Improvement in dextran yield by mineral supplimentation has also been reported by Jeanes et al¹⁶.

2) Effect of Different Nitrogen Sources on the Production of Dextran:

Different nitrogen sources ($\text{NH}_4)_2\text{SO}_4$, NH_4Cl , NH_4NO_3 , Urea) showed a variable effect on the production of dextran (Fig.II). Urea was found to be the more efficient nitrogen source as the dextran production was greatly improved when medium was supplemented with urea. Ammonium nitrate and Ammonium chloride showed the adverse effect as they decreased the dextran production from 35 g/l to 30-32 g/l.

3) Effect of Different Cations on the Production of Dextran:

Fig.III depicts the effect of Mn^{++} , Fe^{++} , Mg^{++} and Zn^{++} on the production of dextran. Mn^{++} and Fe^{++} (upto a concentration of 100 ppm) and Mg^{++} and Zn^{++} (upto 150 ppm) improved the dextran production. Similar observations have been made by Jean¹⁶ and Co-worker¹⁶ who reported that Fe^{++} and Mn^{++} has ~~no~~ significant effect on the dextran formation. ~~and a positive effect.~~

4) Physico-chemical Characteristics

Table-III records the physico-chemical characteristics of various dextran fractions and that of their solutions. Fraction I and II showed similar values for intrinsic viscosity (1.20 and 1.18), specific rotation (+211 and +210) and molecular weights (65,000 and 64800) where for fraction III these values were 0.86, +203 and 30,861 respectively. Variation in viscosity and sp. rotation of these fractions seems to be due to the difference in degree of polymerisation, molecular weight and branching (proportion of 1-6, 1-4 and other linkages). It has been reported that a decrease in proportion of 1-6 linkages results in the increase of

branching and polymer deviates from linearity towards more randomly coiled structure which effect viscosity, solubility and precipitation properties of the polymer.

Specific rotation also depends upon the structural arrangement of the polymer and its has been shown¹ that 1-3 linkages present in the dextran molecule has a rough correlation with specific rotation. The present findings are in accordance with those of Jeanes et al¹ as fraction I and II having a high proportion of 1-3 linkages (Table IV) showed a higher values of specific rotation whereas fraction III (having no 1-3 linkage) showed lower specific rotation.

Chemical analysis showed that all fractions have negligible amounts of nitrogen (0.011 - 0.012%) and ash (0.002 - 0.003%). All fractions were free of any reducing impurity.

Periodate Oxidation.

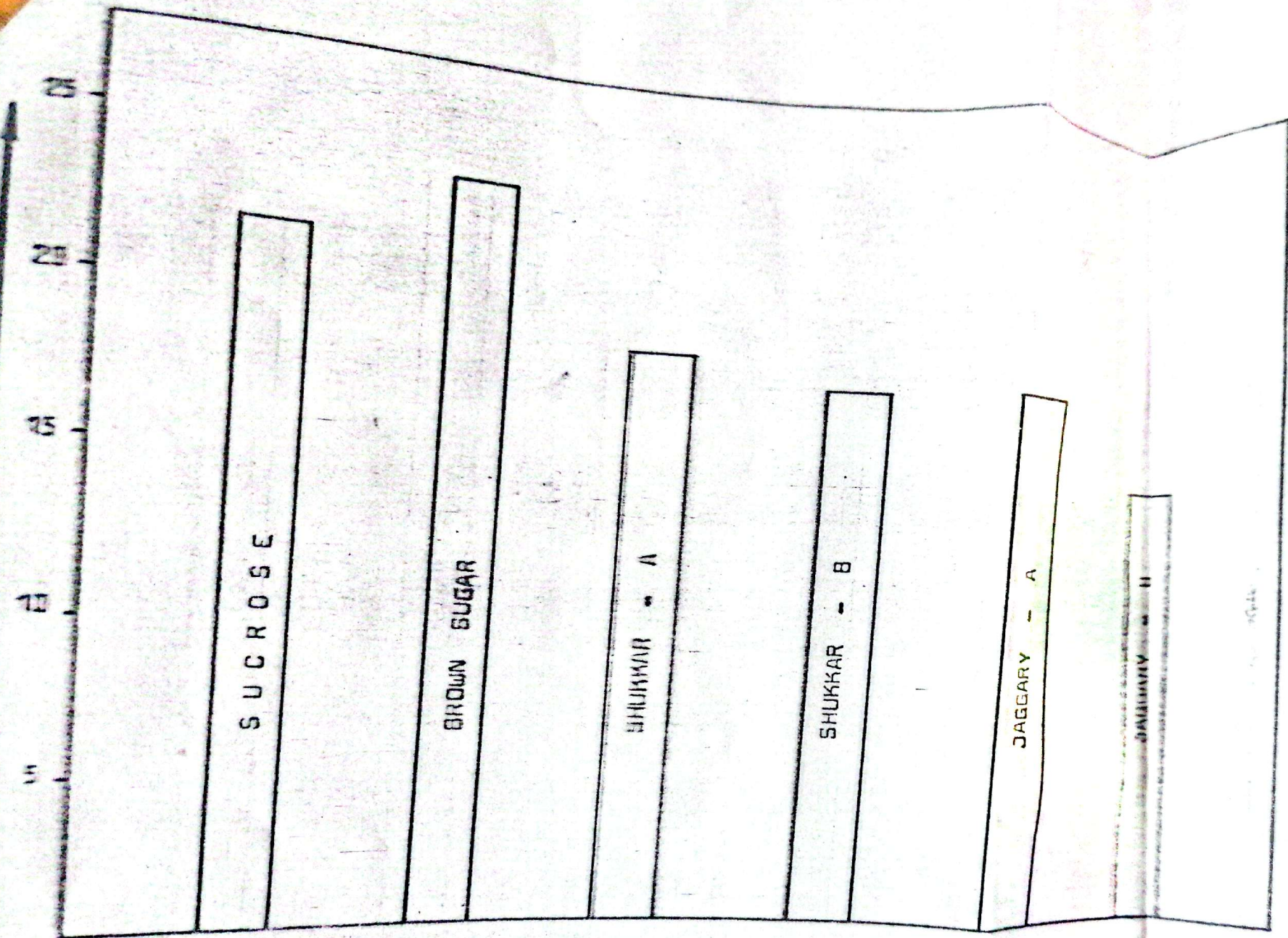
The periodate oxidation determined the percentage of various linkages present in the polysaccharide. All fractions were found to have variable percentage of linkages 1-6 and 1-4 units with the former being dominant (92-97%). Fraction III had no 1-3 linkages whereas this type of linkage was present in fraction I and II to an extent of 2% (Table IV).

The variation in the percentage of different linkages provides an evidence for the branching structure of the molecule. The decreasing content of 1-6 links and increasing content of other links indicate the degree of branching present in the molecule which may affect the other properties such as solubility, precipitation, optical activity etc. of the dextran¹. The higher values of the specific rotation, viscosity and nature of precipitate of fraction I and II (Table-III) also support this fact

as these fractions have lower percentage of 1-6 links
as compared to fraction III.

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Effect of Sucrose and Other Allulose products on the yield of *Leuconostoc mesenteroides* (B₁)

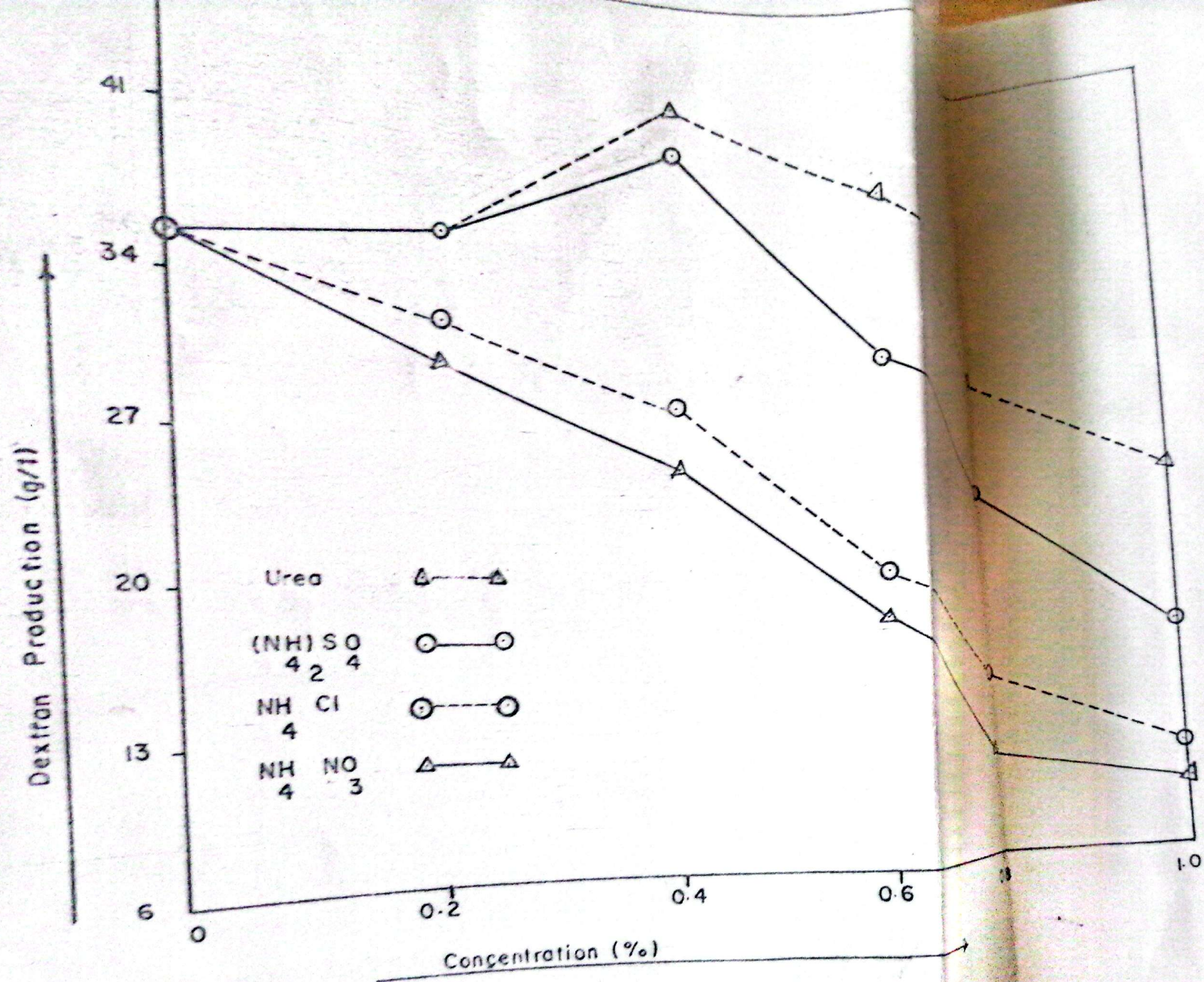


Fig: 21 Effect of Different Nitrogen Sources On The of Dextran By *Leuconostoc mesenteroides* (BI)

Fig: III Effect of Different

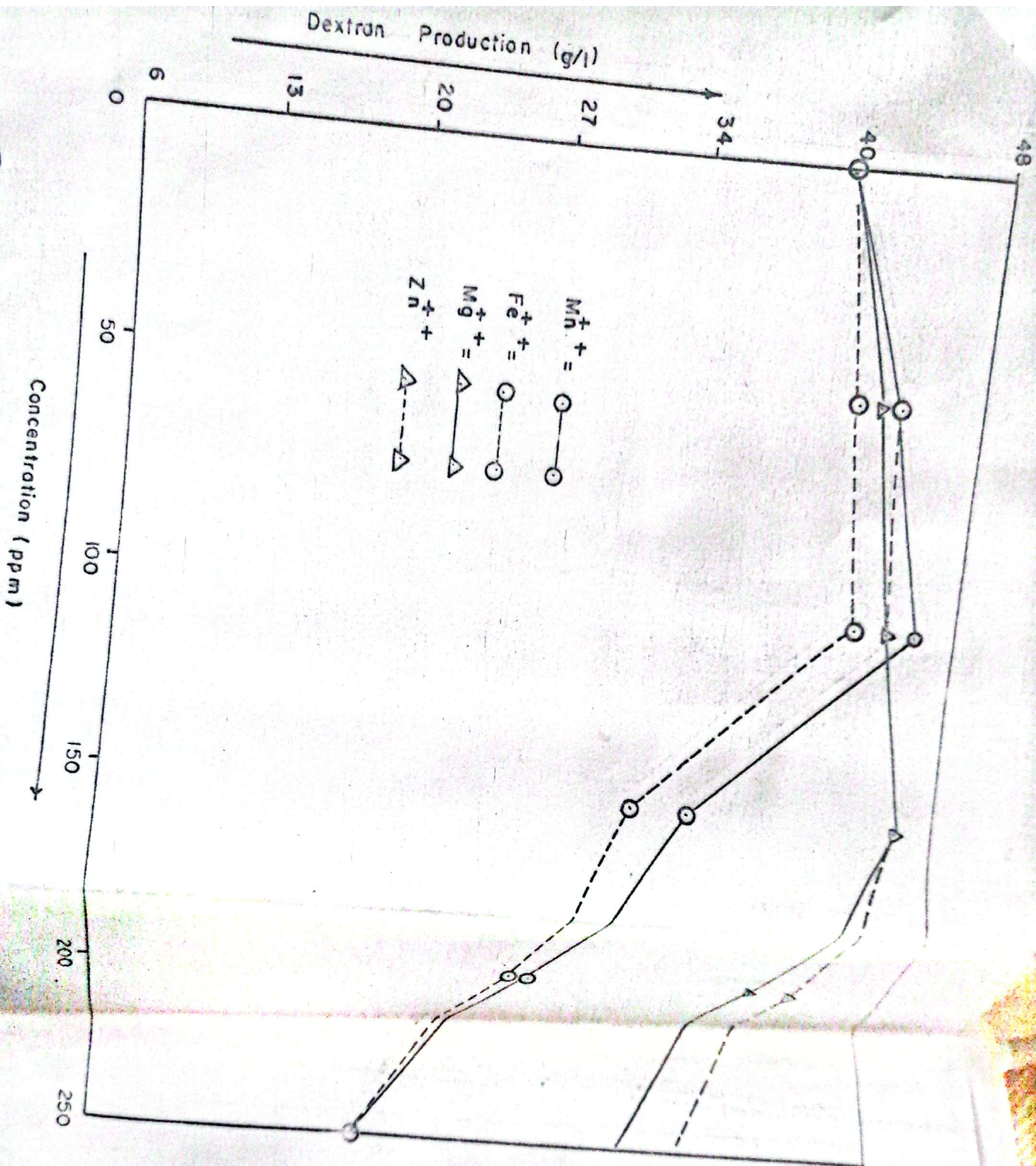


Table - 4x

Chemical Analysis of Different Samples and Allied Products

Samples	Dry Matter %		Reducing sugars %		Nitrogen %		Ash %	
Sucrose	99.20	-	-	-	-	-	1.14	-
Brown sugar	93.20	0.07	-	-	-	-	0.54	-
Shukkar - A	95.66	6.25	-	-	-	-	6.86	-
Shukkar - B	92.73	5.00	-	-	-	-	7.07	-
Jaggery - A	91.71	5.50	-	-	-	-	11.68	-
Jaggery - B	92.96	12.00	-	-	-	-	8.68	-
Glucose	98.10	95.12	-	-	-	-	1.20	-

Table - ^{III}
XIV

Physico-Chemical Characteristics of Dextrane
from *Leuconostoc mesenteroides* (B₁)

Dextran Fractions	% tage of each fraction	Visco- sity at 25°C (η)	Sp. ro- tation 25°C [α] _D	Degree of po- lyme- riza- tion	Mole- cular weight	Characteristics of solution and precipitate		Chemical composition			
						Aqu- eous solu- tion	**** Preci- pitata	Mole- ture %	Nitro- gen %	Ash %	Bene- dict's test
Fraction I	10	1.20	+211	401	65,000	Turbid	Floccu- lant ppt	12.0	0.01	0.003	-tive
Fraction II	78	1.18	+210	400	64,800	Turbid	Floccu- lant ppt	12.5	0.01	0.004	-tive
Fraction III	12	0.86	+203	190	30,861	Turbid	Fine ppt	11.8	0.01	0.002	-tive

* Medium : Brown sugar 15%; Kcl 0.3%; Urea 0.4%; yeast extract 0.1%;
pH 6.8-7.0.

** 1 % Aqueous solution.

*** 0.1 % 1N KOH solution.

**** Precipitated with 95 % ethanol.

Table - ~~xv~~ ^{IV}

Periodate Oxidation of Dextran Fractions
from *Leuconostoc mesenteroides* (B₁)

Dextran Fraction	Moles of periodate reduced/mole of AGU *			Moles of formic acid produced/mole of AGU			Percentage of various linkages **		
	48 hours	96 hours	144 hours	48 hours	96 hours	144 hours	1-6	1-4	1-3 and others
Fraction I	1.80	1.94	1.94	0.888	0.965	0.968	96	2	2
Fraction II	1.70	1.86	1.90	0.880	0.919	0.920	92	6	2
Fraction III	1.84	1.94	1.97	0.890	0.950	0.970	97	3	0

- * AGU stands for Anhydroglucose unit.
 ** Calculated from the values obtained after 144 hours oxidation.